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Suhuan Wu
College of Medicine, Hebei University, Baoding 071000, China.

Siyue Jia
College of Medicine, Hebei University, Baoding 071000, China.

Mengya Shi
College of Medicine, Hebei University, Baoding 071000, China.

Xiaodong Dong
College of Medicine, Hebei University, Baoding 071000, China.

Correspondence
Xiaodong Dong
College of Medicine, Hebei University, Baoding 071000, China.

Study on detection methods for ractopamine

Suhuan Wu, Siyue Jia, Mengya Shi and Xiaodong Dong

Abstract
Ractopamine (RAC) is a kind of phenol amine β_2-agonist, which can significantly increase the lean meat percentage and improve the feed conversion ratio. So RAC as a new growth-promoting feed additive may be used in animals, but when the accumulated dose of RAC exceeds a certain value in our body, it is easy to have some toxic reactions such as trigger muscle tremor, tachycardia, and muscle pain. Therefore, it is extremely important to detect the RAC content for the assurance of consumer health. A number of analytical methods have been developed to provide fast, sensitive, selective and reliable quantification in complex samples. In this article the studies of detection methods for RAC in recent years are reviewed.

Keywords: ractopamine; lean meat; determination; detection; sensor

1. Introduction
Ractopamine (RAC) is a phenethanolamine with β_2-adrenergic agonist properties. It has been originally used as tocolytics, bronchodilators, and heart tonics in human and veterinary medicine. Meanwhile, RAC has been shown to reduce fat deposition through a direct action on adipose tissue and promote muscle growth in livestock [1, 2]. However, the drug residues accumulated in animal tissues may pose a potential risk for consumer health, such as muscular tremors, vomiting, nervousness, and cardiac palpitations. Hence, RAC is not licensed for animal production in many countries [3]. Up-to-now, different analytical methods with a high sensitivity and good selectivity have been developed for the detection of RAC in animal feeds, animal tissues and body liquids. In this paper, the attributes of different analytical technique for the determination of RAC in recent years are reviewed.

2. Analytical Methods
2.1. Colorimetric method: Colorimetry has commonly been used for routine analysis due to its simplicity, low-cost and practicability. It does not require any expensive or sophisticated instruments and the color changes can be even directly observed by the naked eye [4-6]. Recently, some colorimetric systems have been set up to detect various kinds of substances such as DNA, biologically relevant molecules, metal ions, viruses and micro-organisms and so on.

Over the last few decades, the gold nanoparticles (AuNPs) have been widely investigated and applied in molecular catalysis and biosensors due to their unique electrical and optical properties. Typically, the synthesized AuNPs with a size of 13 nm exhibit a specific absorption band around 520 nm while dispersed in liquid media. With the continual aggregation of AuNPs, the increased particle size will cause a red shift in the absorption spectrum which is easy to observe and analyze. Because of the high extinction coefficients and the unique size dependent optical property of AuNPs, AuNP-based colorimetric assays have been widely applied for detecting DNA, proteins, metal ions and small molecules. The reasonable designs of the surface chemistry of AuNPs promote specific interactions between receptors and analytes, leading to a highly selective detection [7, 8]. These methods require a very simple sample preparation process and minimal apparatus investment and can be conducted in the field with portable devices. Therefore, they are very promising in the field of sensor.

Luo *et al*. [9] developed a sensitive method for the colorimetric detection of clenbuterol and RAC using citrate-stabilized AuNPs as probe. The concentration of clenbuterol and RAC could be determined with naked eyes or a UV-vis spectrometer. By optimizing the influence of $NaHSO_4$ and incubation time, clenbuterol could be detected in the linear range of 0.1–4μg/mL with the detection limit of 0.0158μg/mL, and RAC could be detected in the linear range of 1–9μg/mL with the detection limit of 0.0229μg/mL. The proposed method could be successfully applied to detect clenbuterol and RAC in pig urines by a simple pretreatment with excellent

recoveries. The proposed colorimetric assay exhibited good reproducibility and accuracy, providing a simple and rapid method for the analysis of clenbuterol and RAC.

Zhou *et al*. [10] developed a highly selective and sensitive method for colorimetric detection of RAC and salbutamol using AuNPs functionalized with melamine, respectively. The presence of these β-agonists induced the aggregation of AuNPs through hydrogen-bonding interaction that was accompanied by a distinct change in color and optical properties, which could be monitored by a UV–vis spectrophotometer or even naked eyes. This process caused a significant decrease in the absorbance ratio (A_{670nm}/A_{520nm}) of melamine–gold nanoparticles, and the color changed from wine red to blue. The systems exhibited a wide linear range, from 1×10^{-10} mol/L to 5×10^{-7} mol/L with a correlation coefficient of 0.995 for RAC. The detection limit of these β-agonists was as low as 1×10^{-11} mol/L. Particularly, the developed method has been applied to the analysis of real swine feed samples and has achieved satisfactory results.

2.2. HPLC method: High-performance liquid chromatography (HPLC) is a powerful tool that enables the separation of complex mixtures into individual components, and is a highly sensitive and reproducible analytical technique. In recent years, HPLC has been combined with many sensitive detection techniques and has experienced continuous improvement of stationary phases, which have improved its sensitivity and specificity. HPLC is currently widely used for the analysis of drugs and dosage forms with respect to quality control, quantitative determination of active ingredients and impurities, monitoring drug blood concentration in patients, and bioequivalence assessment [11-13].

Du *et al*. [14] developed a simple and sensitive method based on the combination of solid-phase microextraction and HPLC with ultraviolet detection for the simultaneous determination of clenbuterol, salbutamol and RAC in pig samples. The calibration curves were linear over a range of 0.5–50 μg/L for clenbuterol and RAC, and 0.2–20 μg/L for salbutamol. The limits of detection were 0.1 μg/L for clenbuterol, 0.05 μg/L for salbutamol and 0.1μg/L for RAC, respectively. This method exhibited the advantages of simplicity, rapidity and low solvent consumption, and was suitable for the monitoring of β_2-agonists residue in pig samples.

Du *et al*. [15] developed a method for rapid analysis of RAC in porcine muscle and urine. The method was based on the combination of microextraction by packed sorbent (MEPS) and HPLC with ultraviolet detection. Compared with solid-phase extraction, the MEPS procedure required less extraction time, sample volume and consumption of organic solvents. The method demonstrated high linearity within 0.01–2 μg/mL for porcine muscle and urine samples. Accuracies of muscle and urine analyses were 93.9–109.2% and 93.4–105.1%, respectively. The method was applied for rapid analysis of RAC in biological samples.

2.3. Chemiluminescence method: Chemiluminescence (CL) is a well-known and popular analytical method because of its high sensitivity, low detection limit, wide linear working range, and its rapidity, as well as the fact that it can be performed with relatively simple and inexpensive instrumentation, as an excitation source and optical filters are not needed. It has been widely applied in various fields, including clinical diagnosis, biotechnology, pharmacology, food safety, and environmental chemistry [16].

Hu *et al*. [17] proposed a novel CL method for the determination of RAC by combining with flow injection technology. The CL system was based on oxidation of luminol by diperiodatocuprate (III) in the presence of RAC. The diperiodatocuprate (III) was obtained by complexation of copper in the trivalent oxidation state with periodate in a strong alkaline medium. Hereby under the optimum conditions, the relative CL intensity was proportional to the concentration of RAC over the range 1.0×10^{-9} g mL^{-1} to 1.0×10^{-6} g mL^{-1}, with a limit of detection of 3.4×10^{-10} g mL^{-1}. The sensitive method was successfully applied for the determination of RAC in urine samples.

Feng *et al*. [18] developed a novel CL method coupled with flow injection technique for the determination of RAC. It was based on the enhancement of the CL by RAC derived from the CL reaction between luminol and ferricyanide in sodium hydroxide medium. The linear calibration range of the CL intensity with respect to the RAC concentration covered from 4.0×10^{-9} to 8.0×10^{-7} g.mL^{-1}. The detection limit is 2.5×10^{-9} g.mL^{-1}. The method was firstly applied to the determination of RAC in biological samples with satisfactory results.

2.4. SPR methods: Comparing with conventional analytical techniques in measurement of residues in complex samples, the major advantage of surface plasmon resonance (SPR) optical biosensors is the minimal matrix effect on the detector response. Furthermore, SPR sensors are label-free, fast, and highly sensitive and selective. It can be used to monitor interactions between molecules, including large biomolecules such as antibodies, antigens, and proteins or small molecules such as pesticides and veterinary medicines [19, 20].

Lu *et al*. [21] constructed a SPR biosensor inhibition immunoassay for determination of RAC residue in pork by immobilizing RAC derivative on the SPR-2004 biosensor chip. After extraction with perchloric acid, pork sample was cleaned by ethyl acetate and analyzed by SPR-2004 biosensor. The limit of detection was 0.6μg/kg for pork sample. Recoveries of RAC were higher than 80% with relative standard deviations below 10%. Although the same pretreatment was applied for the UPLC–MS/MS, the SPR biosensor showed little matrix interference by constructing pure solution and matrix-match calibration curves. Accordingly, this biosensor was a promising screening instrument to be used for detection of RAC in supermarkets, food factories and food regulatory organizations.

Li *et al*. [22] studied the interaction between the anti-RAC monoclonal antibody and the RAC derivation immobilized on the sensor chip surface with RAC biosensor. They developed a continuous detection method based on the linear response during the association phase. The detection was performed as an inhibitive immunoassay. The mixture of anti-RAC monoclonal antibody and the sample flowed over the surface with RAC derivation was immobilized. The relative response was in inversely proportion to the concentration of RAC. The detection limit was less than 4 μg/L with a detection time of 15 min.

2.5. Electrochemical method: Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. Since RAC contains phenolic hydroxyl group, it should be electrochemical active and could be oxidized at electrode surface, electroanalysis of RAC based on its electro-oxidation has been widely studied. Since the bare electrodes have poor sensitivity and selectivity, the oxidation peak

potential of RAC appears more positive over potential at the bare electrodes, much effort for RAC detection has been devoted to design the modified electrodes to improve the catalytic properties, sensitivity, and selectivity of electrochemical sensors. Numerous materials, such as metal nanoparticles, polymers, carbon nanotubes, fullerenes, graphenes, and enzymes, have been used as modifiers to construct highly sensitive and selective RAC biosensors [23-25].

Wang *et al*. [26] developed a multiplexed electrochemical biosensor for fast and sensitive detection of RAC, salbutamol (SAL) and clenbuterol (CLB) based on reduced grapheme oxide (rGO) and silver–palladium alloy nanoparticles (AgPdNPs). RGO with high conductivity was used as an electrode material to immobilize artificial antigens and amplify electrochemical signal. AgPdNPs were used to label antibodies and generate a strong electrochemical signal in phosphate buffered saline without any other substrates. Screen-printed carbon electrode and competition strategy were adopted to achieve simultaneous detection of RAC, SAL and CLB without cross-talk between adjacent electrodes. Satisfactory results were achieved in pork sample analysis. The designed strategy provided a promising potential in determination of other biological samples.

Zhang *et al*. [27] fabricated a label-free electrochemical immunosensor for sensitive determination of RAC based on Au@Ag_2S core@shell nanoparticles/magnetic chitosan/thionine matrix film (CSMCM). The CSMCM not only enhanced the loading capacity of the biomolecules and stability of the immunosensor, but also provided a favorable microenvironment to maintain the activity of the immobilized biomolecules. Thionine was fabricated into the matrix to increase the conductivity of the CSMCM. Under optimal conditions, the fabricated immunosensor exhibited a linear response with RAC in the concentration range of 0.01–10 ng mL^{-1}, with the low detection limit of 2.5 pg mL^{-1}.

2.6. QCM method: Among various techniques, quartz crystal microbalance (QCM) could measure nanogram-scale changes in mass on the quartz crystal surface based on the Sauerbrey equation by recording its frequency shifts. And QCM has been widely used because of its many advantages such as high sensitivity, low cost, easy installation and inherent ability to monitor analytes in situ and real time. However, the relatively poor selectivity of the QCM technique caused that much effort has been devoted to the development of more specific and selective affinity based recognition layers including antibodies, aptamers, enzymes and also their synthetic equivalents [28, 29].

Considering various artificial receptors used as recognition element in QCM chem/bio sensors, molecularly imprinted polymers (MIPs) are currently most dominant and favored structures that are able to specifically bind a chemical species. Kong *et al*. [30] developed a molecularly imprinted QCM sensor for RAC detection by electrodepositing a poly-*o*-aminothiophenol membrane on an Au electrode surface modified by self-assembled AuNPs. This molecularly imprinted QCM sensor showed good frequency response in RAC binding measurements and the introduction of AuNPs demonstrated performance improvements. Frequency shifts were found to be proportional to concentration of RAC in the range of 2.5×10^{-6} to 1.5×10^{-4} mol L^{-1} with a detection limit of 1.17×10^{-6} mol L^{-1}. This research has combined the advantages of high specific surface area of AuNPs, high selectivity from molecularly imprinted electrodeposited membrane and high sensitivity from quartz crystal microgravimetry. In addition, the modified electrode sensor was successfully applied to determine RAC residues in spiked swine feed samples with satisfactory recoveries ranging from 87.7 to 95.2%.

Chen *et al*. [31] developed an enzyme-amplified magnetic immunosensor for the sensitive and selective detection of RAC in fodder using a QCM. Under optimum conditions, the differences of the QCM frequency were proportional to the concentration of RAC over the range from 0.03 to 25 ng mL^{-1}. The detection limit was 0.01 ng mL^{-1}. Due to its high sensitivity, portability and selectivity, the immunosensor achieved reliable quantification of RAC in real fodder samples and had the potential to become an on-site screening tool to enhance food safety.

2.7. Other methods: In addition to these main approaches mentioned above for RAC detection, still a few special techniques with high sensitivity have been applied. Wang *et al*. [32] determined RAC in pork by using electrochemiluminescence inhibition method combined with molecularly imprinted stir bar sorptive extraction. Wang *et al*. [33] developed an enzyme-assisted and nitrogen-blowing salt-induced solidified floating organic droplet microextraction for determination of clenbuterol and RAC in swine feed via capillary electrophoresis. He *et al*. [34] determined RAC and clenbuterol in feeds by gas chromatography–mass spectrometry. Liu *et al*. [35] synthesized a novel dual-function molecularly imprinted polymer on CdTe/ZnS quantum dots for highly selective and sensitive determination of RAC.

3. Conclusions

The RAC abuse leads to residue in edible animal products and may pose a potential risk for consumer health [36]. To ensure food safety, a rapid and effective method for the determination of RAC would be useful and necessary. This review has highlighted the significant developments in rapid and alternative techniques for the detection of RAC in recent years. New developed methods with better sensitivity and specificity, along with more simplicity and lower cost is still the future direction.

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